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Cloning and Chromosomal Assignment of the Porcine Interleukin-2 Receptor Alpha (IL-2Rα) Gene

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ABSTRACT. Porcine genomic DNA encoding a 55 kDa subunit of interleukin-2 receptor (IL-2R), which is termed α chain (IL-2Rα), was cloned by repeated plaque hybridization using IL-2Rα cDNA as a probe. Two different lambda phage clones, one of which encoded exon 1 and the 5'-upstream flanking region of IL-2Rα gene and another encoded the sequence from exon 2 to exon 8, were isolated. By analysis of the 5'-upstream region of the gene, putative binding motifs for transcription factors such as GATA family proteins, Ikaros, NF-κB, NF-IL2Rα and SRF, were found as described in human, murine and bovine genes. Two additional motifs for STAT4 binding were also found in this region. Moreover, using the FISH technique, we assigned the porcine IL-2Rα locus to the distal end of the long arm of chromosome 10 (10q6-qter) where the vimentin gene had been assigned nearby.

KEY WORDS: genome mapping, interleukin(IL)-2 receptor, swine.

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Interleukin (IL)-2 receptor (IL-2R), is a heterotrimeric complex composed of non-covalently associated 55, 75 and 65 kDa subunits designated α, *β* and *γ* chains, respectively [3, 26]. The *β* and *γ* chains, which are the components shared with IL-15R [8], are constitutively expressed on the surface of a wide variety of blood cells, and form an intermediate affinity receptor for IL-2 (Kd=1 × 10^{-9}). In contrast, the α chain, which is well known as an affinity converter molecule, is mainly expressed on peripheral mature T cells after antigenic/mitogenic activation *in vitro* and *in vivo* [17]. Although the α chain does not induce any intracellular signal alone, it increases the receptor affinity at least 100-fold (Kd= 1 × 10^{-11}) by associating with a pre-existing *βγ* receptor complex mentioned above [17]. Consequently, such affinity conversion results in the drastic enhancement of IL-2-dependent proliferation of responding T cells. The up-regulation of IL-2Rα gene transcription is, therefore, one of the keys for T cells to exert their immunological activities.

In human and mice, the regulatory mechanism of IL-2Rα gene expression has been investigated extensively and several response elements have been identified in the 5'-upstream region of the genes [4, 11, 12, 21, 24]. Despite these reports, the mechanism of basal/induced expression of IL-2Rα has not been completely elucidated. Recently, we reported cDNA cloning of porcine IL-2Rα gene and the detection of mRNA in mitogen-activated porcine peripheral blood mononuclear cells (PBMCs) [13]. In pigs, IL-2Rα is the only activation/differentiation marker of lymphoid cells that was classified by the International Swine CD Workshop [22]. Therefore, understanding of the molecular basis on the transcriptional regulation of this gene would provide useful information of the activation and the maturation of swine lymphocytes. To explore the mechanism, we isolated genomic clones that encoded porcine IL-2Rα and determined their nucleotide sequences including the promoter/enhancer region (Genbank accession No. AF052037 and AF036005). Moreover, we performed the chromosomal mapping of the IL-2Rα locus using a fluorescence *in situ* hybridization (FISH) technique for the development of comparative map of the mammalian chromosomes.

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MATERIALS AND METHODS

Screening of a porcine genomic DNA library: Lambda (λ) FIX II phage clones encoding porcine IL-2Rα gene were isolated from approximately 1 × 10^6 clones of a porcine genomic DNA library (Stratagene, La Jolla, CA) by repeated plaque hybridization as described by Sambrook *et al.* [23]. For the preparation of a hybridization probe, a *Pvu* II fragment (1.1 kbp) of porcine IL-2Rα cDNA clone, p2r, was labeled with ^{32}P-α-deoxy CTP (NEN, Boston, MA) using the Megaprime DNA labeling system (Amersham Pharmacia Biotech, Uppsala, Sweden). Six positive clones were selected by restriction endonuclease digestion and Southern blot hybridization. These were then subjected to DNA sequencing analysis after subcloning of their inserts into a plasmid vector, pT7 (Invitrogen, Leek, the Netherlands) or pBluescript II SK(-) (Stratagene) .

DNA sequencing: DNA sequencing analysis was performed with the ThermoSequenase DNA Sequencing Kit (Amersham Pharmacia Biotech) using an automatic DNA sequencer, the A.L.F. express (Amersham Pharmacia Biotech) as described in manufacturer's protocol.

Primer extension analysis: Primer extension analysis was performed as described previously [18] with slight modifications. In brief, Cy5-labeled oligonucleotide primers (CyP2REX1; 5'-GCCATGCAGCCGGGTATC-3' and CyP2U1AS; 5'-AGCTGCCAGCTACGACGTG-3'), which were complementary to the flanking sequences of the translation start site, were annealed with 5 μg of RNA prepared from con A-stimulated porcine PBMCs in a buffer containing 125 mM KCl, 25 mM tris-HCl, pH 8.8, 10 mM $MgCl_2$, 0.25% Triton X-100, 10 mM dithiothreitol, and 20 U of RNasin Ribonuclease Inhibitor (Promega, Madison, WI). After the addition of dATP, dCTP, dTTP and deaza-dGTP (1 mM each), a primer extension reaction was carried out with 20 U of moloney murine leukemia virus (M-MLV) reverse transcriptase (Gibco BRL, Gaithersburg, MD) at 48°C, for 1 hr. A reaction without the enzyme was carried out for the negative control experiment. After RNase treatment, the synthesized DNA was extracted with phenol-chloroform (1:1), precipitated in ethanol and resolved in 4 μl of distilled water. The same volume of stop buffer from the AutoRead Sequencing Kit (Amersham Pharmacia Biotech) was added, then, the mixture was loaded into the gel of the A.L.F. DNA sequencer. The electrophoresis pattern was analyzed in the aid of the program, Fragment Manager Ver.1.2 (Amersham Pharmacia Biotech).

Fluorescence in situ hybridization (FISH): For the assignment of the fragments to porcine chromosomes, lymphocytes were cultured as described elsewhere [20, 31] to prepare chromosome-spreads. For the probe, a mixture of DNA of the phage clones containing a part of IL-2Rα gene was labeled with biotin-16-dUTP using a nick translating kit (Boehringer Mannheim Co., Germany). Two-hundred-fifty nanograms of the labeled probe DNA, 5 μg of porcine Cot-5 DNA (repetitive sequence-enriched DNA), and 3 μg of herring sperm DNA was dissolved in a 10 μl of formamide and denatured at 75°C for 10 min, followed by mixing with 10 μl of the hybridization buffer (4x SSC, 100 mM phosphate buffer, pH 7.0, 20% dextran sulfate, 2x Denhardt's solution, and 0.2% SDS). The resulting mixture (20 μl) was incubated for 15 min at 37°C to reduce the hybridization signals of repetitive sequences, before applying to chromosome spreads, then with chromosome spreads for 16 hrs at 37°C to hybridize the probe DNA to chromosomal DNA. After hybridization, the spreads were rinsed and processed for detection of the hybridization signals on R-banded chromosomes as described previously [2].

RESULTS

Genomic DNA cloning: Two different groups of λ FIX II phage clones, one of which had an insert encoding the first exon of porcine IL-2Rα gene and its flanking upstream sequence, and another containing the sequence extending from exon 2 to exon 8, were isolated from approximately one million of independent clones of a λ phage library. Restriction enzyme maps and exon/intron structures in *Not* I fragments of representative isolates designated clone U21 and clone 101 are shown in Fig. 1A. The lengths of the exons (exon 1–8) appeared to be 288 bp, 192 bp, 114 bp, 228 bp, 72 bp, 72 bp, 67 bp and >400 bp, respectively. A *Bam*H I fragment (1247 bp) of clone U21, which encoded the proximal flanking region of exon 1, was cloned into pBluescript II and subjected to DNA sequencing analysis (Fig. 1B). In contrast to other mammalian IL-2Rα genes, a single TATA-box like sequence (TATATTT) was observed in the porcine gene at the position of -260 bp with respect to ATG translation start site immediately downstream of the short poly d(A) tract. By further anal-

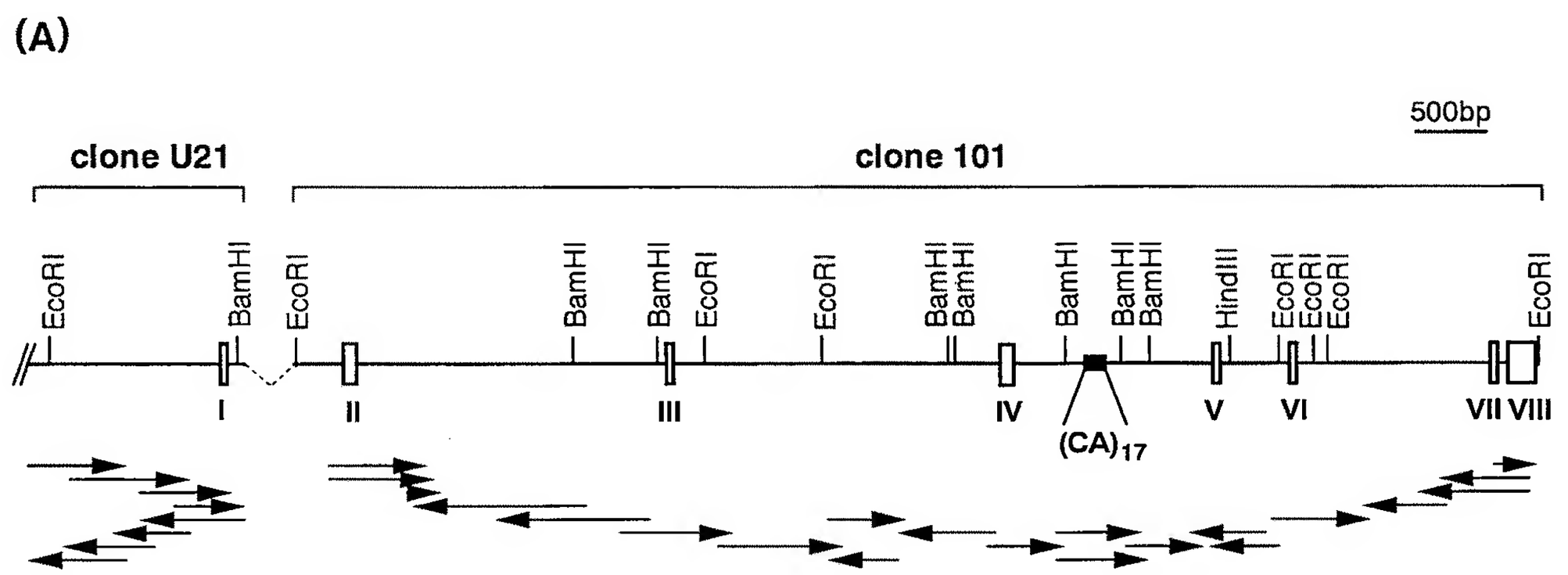

Fig. 1. The genomic structure (A) and 5'-upstream sequence (B) of porcine IL-2Rα gene. (A) The restriction endonuclease map of porcine IL-2Rα gene obtained from clone U21 and clone 101 is shown. Each exon was indicated as a box. The direction and extent of sequencing are indicated by horizontal arrows. The size of exon 8 was estimated from the distance to the first polyadenylation signal. (B) The nucleotide sequence of exon 1 (upper case) and its flanking region (lower case) are shown. The position of the first adenine of the translation start site (ATG) is indicated as +1. The consensus motifs for GATA family proteins, NRE-2, SRF, Ikaros, NF-κB, NF-IL2Rα and STAT4 were underlined. The poly d(A) tract is also underlined. A TATA box sequence is indicated by a box. The sequences corresponding to the complementary oligonucleotide primers for primer extension are shown in boldfaced italics. The determined positions of the transcription initiation sites are indicated by arrows.

(B)

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gaattctgag tatggatcag tttgctgctt ccttttcaat gacctgaata -1196
ttggagaccc catcctgacg cccactagac tttgaaaaaa tgttattgaa -1146
atttagttca tctacaatgt tgtgttaatt tctgctatat agcaaagtga -1196
tttgaataca tacacacaca cacatatgta tataatggaa aaggctatga -1046
aagaaaagac tatatagata gtcatatgta agactgtgaa agagaagata  -996
                    GATA
tatatatata acatggaaaa cacaaggaaa agtatatacg tatatattct  -946
ttttcatagt ccttctccgt catggttttat cacgatactg aacatagctc  -896
                                GATA
cctgtgctct acagtaggac ctgggcattt atccatctat atgcactagt  -846
ttgcatctgc taatcccaaa ctcccgatcc atccctcccc tcccctctcc  -796
ccctcggcaa ccccaagtct gttctctagg tctgcaagtc cgtgtctgtt  -746
tcctcgataa gttcatttgt gtcatatttt agattccacg cggaagtggt  -696
 STAT4
atcacatggt atgtgtcctt ctctgacgga cttcgcttag tgtggtcatc  -646
tccaggtccc tccagattgc tgcacatttt ttaaagagat ccaggcagac  -596
agctttagga aattcccatt tctggccaag actgctcttc cacacttttg  -546

      NF-κB  Ikaros

gcatggcctt gtccccatgt cctgctgact cctctgggag ctgcaagtct  -496
ggggaaggac ctcagcagtt tcacggtgac atcagtctgt gaaaccctag  -446
                          STAT4
atgagcccac tgccaagaag tgcttgctca ccctccttca acggcagagg  -396

     NRE-2                              NF-IL2Rα

gaatctccct ctccttttttt gggggggaagc tgaagaaagg attcataaat  -346

 NF-κB          SRF

gaaccccagt gctcctcacc aacctcagcc cacacctccc agcaactgaa  -296

cttgaaaaaa acctggttaa aaccacttcc tatatttgaa actagagaag  -246

         poly d(A)                    TATA box

                                  ↱
agagtgctat aggcactatt tcctggctga accgaccagg ctgttactta  -196

                ↱
gacgcagagc cagtgcagac agccccagag cctggacgga cgggcgagga  -146
tgacagcctg agcggaggga gccctcatcc ccgtcctctc ggcgagatgc  -96
taaccagagg ctgacagggc ccggcatccg acggagaaac tcccctcctc  -46

ctggccccac gtcgtagctg gcagctgatc ccaaggacca ggaggATGGA    5
             CyP2U1AS                              +1
GCCGAGCTTG CTGATGTGGG GATTCTTCAC ATTCACCATG ATACCCGGCT   55
                                             CyP2REX1
GCATGGCAGg taaggggtca caggggccct tggagtcttg tgggggggct  105
aaggatgtcg actctcccgg gcacagaatc agagagggca cctgagtaga  155
cagcagaggg aaggggggttg gatcc                            180
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ysis of conserved motifs for transcription factors, several binding motifs such as for NF-κB, Ikaros and the serum response factor (SRF) were found in the region as indicated (Fig. 1B). It was noteworthy that other additional motifs for an IL-12-dependent transcription factor, signal transduction activating transcription factor-4 (STAT4), and a poorly characterized, IL-2Rα gene specific transcription factor, NF-IL2Rα [1, 16, 27], were also observed in this region. More-

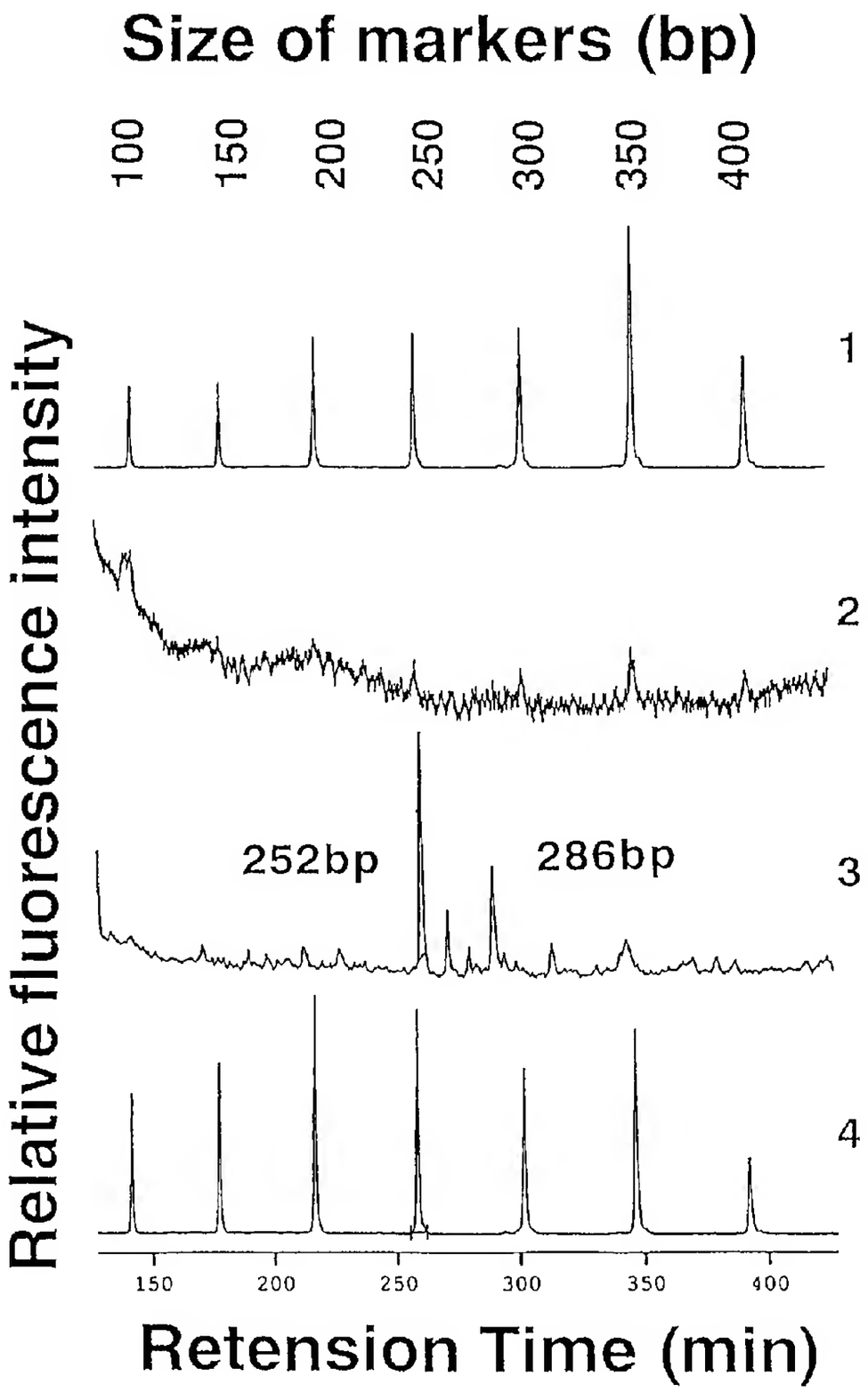

Fig. 2. Determination of the transcription start site of porcine IL-2Rα gene. The primer extension products were obtained using RNA from con-A activated porcine PBMCs as a template, and the fragment size was analyzed on the gel of an automated fluorescence DNA sequencer, A.L.F. express. The electrophoresis patterns of Cy5-labeled molecular size standard (lane 1 and 4), the enzyme omitted control (lane 2) and the primer extension products (lane 3) are shown. The retention times of the products are indicated at the bottom.

over, an *Eco*R I fragment (approximately 8.5 kbp) of clone 101 was transferred into pT7 vector, and subjected to the nucleotide sequencing. A dinucleotide $(CA)_{17}$ repeat sequence, which would be useful for PCR amplification and linkage mapping of the gene, was found between exon 4 and 5 (intron 4). The sequence data described here have been submitted to Genbank and have assigned the accession numbers AF052037 and AF036005.

Determination of transcription initiation site in porcine IL-2Rα gene: Primer extension analysis was performed to determine the transcription start site by using fluorescence-labeled primers and an automated DNA sequencer. Two major extension products were detected at the positions of 252 and 286 base long after the extension with CyP2REX1 (Fig. 2), and of 171 and 205 base long with CyP2U1AS (data not shown), respectively. These results demonstrated the transcription start sites being at 35 and 69 bp downstream from the 3'-end of TATA box, which seem to be similar with the positions previously reported on human [10, 14] and murine [5, 6] genes. The 252 bp product generated by the reaction with CyP2REX1 primer might indicate an alternative transcription start site.

Genomic mapping of porcine IL-2Rα locus: Fluorescence *in situ* hybridization was performed using a mixture of both λ phage DNAs (U21 and 101) as a probe. More than 150 chromosomes spreads were inspected under a fluorescence microscope. A representative chromosome spread possessing hybridization signals is presented in Fig. 3. Hybridization signals were consistently observed at the telomeric region of swine chromosome 10q-arm. When 96 chromosome spreads showing signals were scored for the precise localization on the chromosome, it was found that IL-2Rα gene resided in the region of swine chromosome 10q6-qter (Fig. 3B).

DISCUSSION

Two different types of λ clones encoding genomic sequences for porcine IL-2Rα were isolated, and the nucleotide sequences of representative clones were determined as shown in Fig. 1. Similar to other mammalian IL-2Rα genes [14, 25], the porcine IL-2Rα gene was composed of eight exons. However, since intron 1, the size of which was inferred to be approximately 38 kbp from analogy to the human counterpart [25], is not clarified completely, it is incapable of determining the entire size of IL-2Rα locus. By screening of a BAC library of porcine genomic DNA by PCR, we isolated a BAC clone that possessed a 125 kbp genomic insert including the full sequence of intron 1 (data not shown).

Some groups have identified proximal and distal responding elements in the 5'-flanking region of human and murine IL-2Rα genes, which are critical for both positive and negative regulation [4, 11, 12, 21, 24]. As shown in Fig. 1B, several binding motifs for NF-κB [16], SRF [11], GATA proteins [19], Ikaros [7] and a poorly defined protein, NF-IL2Rα [1, 16, 27] were found in the upstream sequence of porcine IL-2Rα gene. These findings show the similarity of this sequence with the proximal element in other examined mammalian genes. Although the precise mechanism of IL-2Rα expression has not been elucidated, this region has been reported to be essential for antigen- and IL-1-induced expression of IL-2Rα [21, 24]. In our preliminary experiment using reporter plasmids carrying different regions of the promoter sequence, it was observed that the NF-IL2Rα motif contributed to the expression of reporter protein at higher extent than the NF-κB motif in mitogen-activated murine T-lymphoma cells (unpublished data). This observation is consistent with the results of other previous reports [16, 27], thus, it is suggested that a common, NF-IL2Rα-dependent mechanism may exist in the transcriptional regulation of these IL-2Rα genes.

In addition to these motifs, we revealed two potent STAT4-

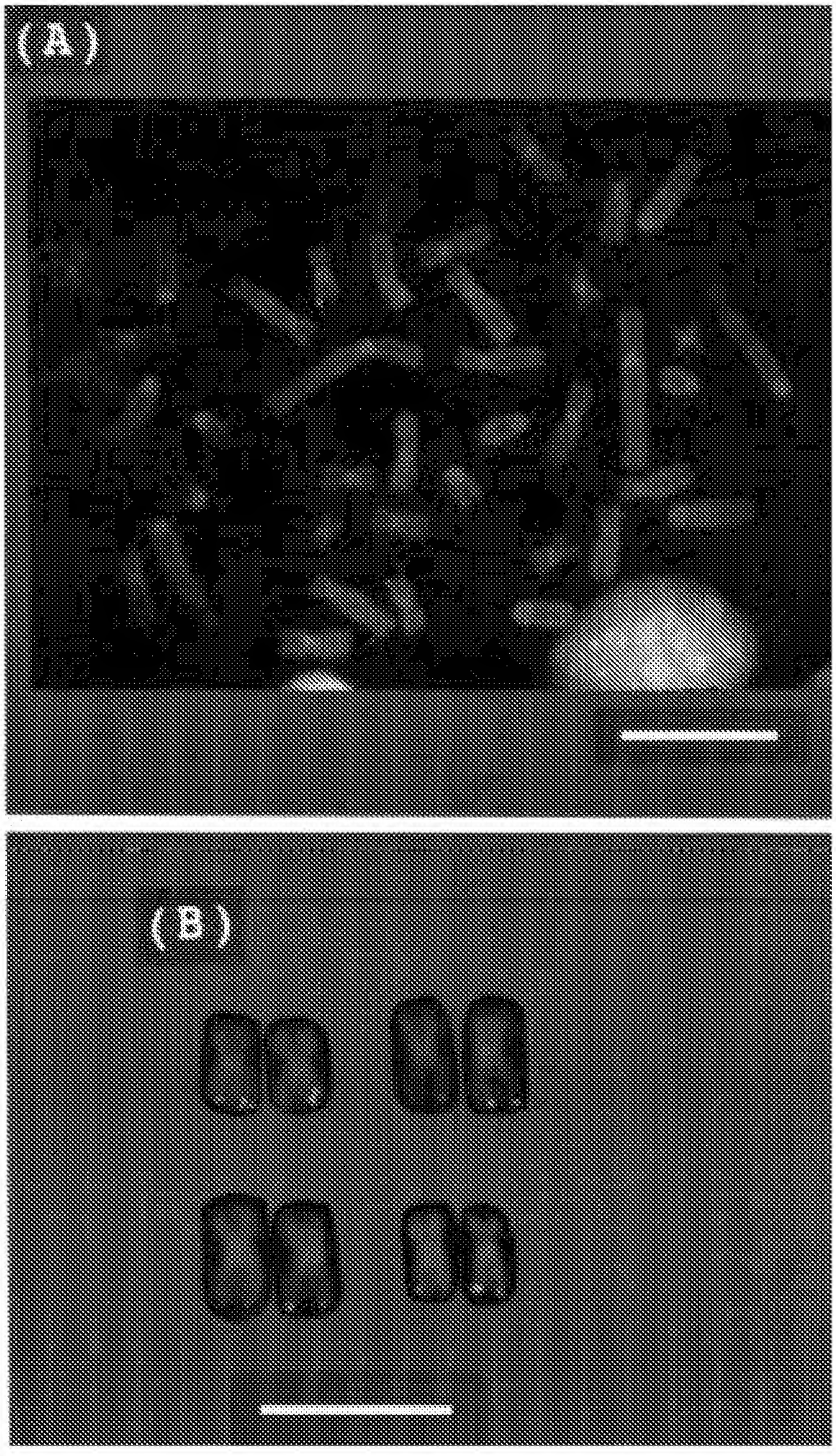

Fig. 3. FISH on swine chromosomes using the lambda phage DNAs containing a part of the swine IL-2Rα gene as a probe. The probe was labeled with biotin and hybridization was visualized with the FITC-labeled streptavidin/biotinylated anti-streptavidin antibody system. Additonally, the chromosomes were stained with propidium iodide to induce simultaneous R-banding. Panel (A) shows a representative chromosome spread; panel (B), four chromosomes 10 tagged with fluorescence signals. The white scale bar is 10 μm.

binding sequences (TTTCACGGTG; from –476 to –467 and TTTCCTCGAT; from –746 to –736), which were not referred to in previous reports. To our knowledge, STAT4 can be activated by only IL-12, which has been reported to be a pivotal cytokine in cell-mediated immunity. Yanagida *et al.* [30] reported that IL-12 induced IL-2Rα expression in Th1 cells, but not in Th2 cells, after antigenic stimulation. Hence our observation suggests the possibility that IL-12 directly regulates the expression of IL-2Rα through STAT4-activating mechanism in Th1 cells. On the other hand, Yamamoto *et al.* [29] demonstrated that STAT4 bound to GAS-like motifs, which were observed in the distal region of IL-2Rα promoter. Thus, crucial sites for STAT4 binding and accurate mechanisms of IL-12-dependent expression of IL-2Rα gene remain to be elucidated.

By primer extension analysis, we examined the initiation site of the IL-2Rα gene transcription. In our data, two major initiation sites were identified at 35 bp and 69 bp downstream from the TATA box. Interestingly, duplicated TATA box-like sequences and multiple initiation sites have commonly been observed among other reported mammalian genes [5, 14, 25, 33]. In human gene, for example, Ishida *et al.* [10] have found two TATA box-like sequences in the proximal region (5'- and 3'-TATA boxes) and demonstrated the existence of multiple initiation sites at approximately 30 bp and 70 bp downstream from the 3'-TATA box. Although the biological importance of such multiplicity in IL-2Rα gene transcription is not known, the TATA box identified in the porcine IL-2Rα gene seems functionally equivalent to the 3'-TATA box of other mammalian genes, which controls the transcription from, at least, two different initiation sites.

FISH analysis data demonstrated that IL-2Rα gene resided in the region of swine chromosome 10q6-qter. In this region, the vimentin gene has been localized nearby [32]. The synteny between IL-2Rα and vimentin genes in the swine chromosome 10 has been conserved in human chromosome 10 [15], murine chromosome 2 [28] and bovine chromosome 13 [33]. However, since the positional relationship of IL-2Rα and vimentin genes has not yet been clarified, additional genes on swine chromosome 10q-arm, which is identified on human chromosome 10 by Zoo-Fish [9], or two-color hybridization of IL-2Rα and vimentin genes on the same chromosome spread is necessary to determine the relationships in the above synthenic group.

In conclusion, we cloned the genomic sequence of porcine IL-2Rα and demonstrated the structure of promoter/enhancer region and chromosomal mapping of the locus. Since IL-2Rα is the only activation/differentiation marker that has been certified on swine lymphoid cells, our data provide useful information to understand the mechanism of lymphocyte maturation in pigs.

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REFERENCES

1. Algarte, M., Lecine, P., Costello, R., Plet, A., Olive, D. and Imbert, J. 1995. *In vivo* regulation of interleukin-2 receptor α gene transcription by the coordinated binding of constitutive and inducible factors in human primary T cells. *EMBO J.* 14: 5060–5072.
2. Awata, T., Yamakuchi, M., Kumagai, M. and Yasue, H. 1995. Assignment of the tenascin gene (HXB) to swine chromosome 1q21.1–q21.3 by fluorescence in situ hybridization. *Cytogenet. Cell Genet.* 69: 33–34.
3. Cao, X., Kozak, C. A., Liu, Y. J., Noguchi, M., O'Connell, E. and Leonard, W. J. 1993. Characterization of cDNA encoding the murine interleukin 2 receptor (IL-2R) γ chain: Chromosomal mapping and tissue specificity of IL-2R γ chain expression. *Proc. Natl. Acad. Sci. U.S.A.* 90: 8464–8468.
4. Cross, S. L., Feinberg, M., Wolf, J. B., Holbrook, N. J., Wong-Staal, F. and Leonard, W. J. 1987. Regulation of the human interleukin-2 receptor α chain promoter: Activation of a non-functional promoter by the transactivator gene of HTLV-1. *Cell* 49: 47–56.
5. Froussard, P., Chastagner, P., Somme G., Abadie, A., Greene, W., Theze, J. and Longacre, S. 1988. p55 IL-2 receptor mRNA precursors in murine T lymphocyte nuclei. *J. Immunol.* 141: 1358–1364.
6. Froussard, P., Jankovic, D. L., Chastangner, P. and Theze, J. 1991. Induction of mouse p55 interleukin-2 receptor gene expression by IL-2 and IL-4 and characterization of its transcription initiation sites. *Mol. Immunol.* 28: 87–93.
7. Georgopoulos, K., Moore, D. D. and Derfler, B. 1992. Ikaros, an early lymphoid-specific transcription factor and a putative mediator for T cell commitment. *Science* 258: 808–812.
8. Giri, J. G., Ahdieh, M., Eisenman, J., Shanebeck, K., Grabstein, K., Kumaki, S., Namen, A., Park, L. S., Cosman, D. and Anderson D. 1994. Utilization of the β and γchains of the IL-2 receptor by the novel cytokine IL-15. *EMBO J.* 13: 2822–2830.
9. Goureau, A., Yerle, M., Schmitz, A., Riquet, J., Milan, D., Pinton, P., Frelat, G. and Gellin, J. 1996. Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. *Genomics* 36: 252–262.
10. Ishida, N., Kanamori, H., Noma, T., Nikaido, T., Sabe, H., Suzuki, N., Shimizu, A. and Honjo, T. 1985. Molecular cloning and structure of the human interleukin 2 receptor gene. *Nucleic Acids Res.* 13: 7579–7589.
11. John, S., Reeves, R. B., Lin, J. X., Child, R., Leiden, J. M., Thompson, C. B. and Leonard, W. J. 1995. Regulation of cell-type-specific interleukin-2 receptor α-chain gene expression: Potential role of physical interactions between Elf-1, HMG-I(Y), and NF-κB family protein. *Mol. Cell Biol.* 15: 1786–1796.
12. John, S., Robbins, C. M. and Leonard, W. J. 1996. An IL-2 response element in the human IL-2 receptor α chain promoter is a composite element that binds Stat5, Elf-1, HMG-I(Y) and a GATA family protein. *EMBO J.* 15: 5627–5635.
13. Kokuho, T., Uchimura, A. and Inumaru, S. 1997. cDNA cloning of porcine interleukin-2 receptor-α gene. *Immunol. Cell Biol.* 75: 515–518.
14. Leonard, W. J., Depper, J. M., Kanehisa, M., Kronke, M., Peffer, N., Svetlik, P. B., Sullivan, M. and Greene, W. C. 1985.

Structure of the human interleukin-2 receptor gene. *Science* 230: 633–639.
15. Leonard, W. J., Donlon, T. A., Lebo, R. V. and Greene, W. C. 1985. Localization of the gene encoding the human interleukin-2 receptor on chromosome 10. *Science* 228: 1547–1548.
16. Lowenthal, J. W., Ballard, D. W., Bohnlein, E. and Greene,W. C. 1989. Tumour necrosis factor α induces proteins that bind specifically to κB-like enhancer elements and regulate interleukin 2 receptor α-chain gene expression in primary human T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 86: 2331–2335.
17. Minami, Y., Kono, T., Miyazaki, T. and Taniguchi, T. 1993. The IL-2 receptor complex: Its structure, function, and target genes. *Annu. Rev. Immunol.* 11: 245–267.
18. Myohanen, S. and Wahlfors, J. 1993. Automated fluorescent primer extention. *BioTechniques* 14: 16–17.
19. Orkin, S. H. 1992. GATA-binding transcription factors in hematopoietic cells. *Blood* 80: 575–581.
20. Pequignot, E. V. 1992. *In situ* Hybridization: A Practical Approach (Willkinson, D. G. ed.), Oxford Univ. Press, New York.
21. Plaetinck, G., Combe, M. C., Corthesy, P., Sperisen, P., Kanamori, H., Honjo, T. and Nabholz, M. 1990. Control of IL-2 receptor-α expression by IL-1, tumor necrosis factor, and IL-2. Complex regulation via elements in the 5' flanking region. *J. Immunol.* 145: 3340–3347.
22. Saalmuller, A. 1996. Characterization of swine leukocyte differentiation antigens. *Immunol. Today* 17: 352–354.
23. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: a Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY.
24. Sperisen, P., Wang, S. M., Soldaini, E., Pla, M., Rusterhorz, C., Bucher, P., Corthesy, P., Reinchenbach, P. and Nabholz, M. 1995. Mouse interleukin-2 receptor α gene expression. *J. Biol. Chem.* 270: 10743–10753.
25. Suzuki, N., Matsunami, N., Kanamori, H., Ishida, N., Shimizu, A., Yaoita, Y., Nikaido, T. and Honjo, T. 1987. The human IL-2 receptor gene contains a positive regulatory element that functions in cultured cells and cell-free extracts. *J. Biol. Chem.* 262: 5079–5086.
26. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. and Sugamura, K. 1992. Cloning of the γ chain of the human IL-2 receptor. *Science* 257: 379–382.
27. Toledano, M. B., Roman, D. G., Halden, N. F., Lin, B. B. and Leonard, W. J. 1990. The same target sequences are differently important for activation of the interleukin-2 receptor α-chain gene in two distinct T-cell lines. *Proc. Natl. Acad. Sci. U.S.A.* 87: 1830–1834.
28. Webb, G. C., Campbell, H. D., Lee, J. S. and Young, I. G. 1990. Mapping the gene for murine T-cell growth factor, IL-2, to bands B-C on chromosome 3 and for the alpha chain of the IL-2 receptor, IL-2Rα, to bands A2-A3 on chromosome 2. *Cytogenet. Cell Genet.* 54: 164–168.
29. Yamamoto, K., Miura, O., Hirosawa, S. and Miyasaka, N. 1997. Binding sequence of STAT4: STAT4 complex recognizes the IFN-γ activation site (GAS)-like sequence (T/A)TTCC(C/G)GGAA(T/A). *Biochem. Biophys. Res. Comm.* 233: 126–132.
30. Yanagida, T., Kato, T., Igarashi, O., Inoue, T. and Nariuchi, H. 1994. Second signal activity of IL-12 on the proliferation and IL-2R expession of T helper cell-1 clone. *J. Immunol.* 152: 4919–4928.
31. Yasue, H. and Ishibashi, M. 1982. The oncogenicity of avian adenovirus III: *In situ* hybridization of tumor line cells localized a large number of a virocellular sequences in few chomosomes. *Virology* 116: 99–115.
32. Yerle, M., Lahbib-Mansais, Y., Pinton, P., Robic, A., Gouteau, A., Milan, D. and Gellin, J. 1997. The cytogenetic map of the domestic pig. *Mamm. Genome* 8: 592–607.
33. Yoo, J., Ponce de Leon, F. A., Stone, R. T. and Beattie, C. W. 1995. Cloning and chomosomal assignment of the bovine interleukin-2 receptor alpha (IL-2Rα) gene. *Mamm. Genome* 6: 751–753.